AN IMPROVED AND RAPID TEST FOR INDOL IN BROTH CULTURES AND FOR THE PRESENCE OF THIS SUBSTANCE IN MEAT-SUGAR-FREE BROTH.*

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IN my previous paper read before the Pathological Society of Philadelphia, March 14, 1907, entitled "Contributions to the Differentiation of *B. coli communis* from Allied Species," we had the opportunity of bringing out some points and reactions as well as some tests characteristic of *B. coli* by means of which this organism could be differentiated from the rest of the Colon group. When I say, from the Colon group, I wish to state again that, based upon our results and observations, *B. coli communis* presents some constant biological features peculiar to itself, by means of which the differentiation can be easily accomplished. As a matter of convenience, these reactions were named "Test 1," "Test 2," and "Test 3," respectively.

Test 1 is a negative test. When about $\frac{1}{4}$ c.c. of sterile dextrose broth is boiled for a few minutes in about 5 c.c. of a 10 per cent sodium hydroxide solution, a light yellow canary color is produced. Similar treatment of a 48-hour-old culture of *B. Coli* produces exactly the same result, whereas with allied species a pinkish coloration is imparted to the liquid on standing from 5 to 15 minutes.

Test 2 consists in a bright purple or pinkish coloration produced by *B. coli* when about 1 c.c. of a 10 per cent sodium hydroxide solution and about 1 c.c. of a 50 per cent sulphuric acid solution are added to the culture; cultures of the saccharolytic group produce no such reaction. A study of the nature of this reaction has proven it to be very closely connected with indol, or at least with some derivative of indican.

Test 3 consists in the inability of *B. coli* to exhaust the sugar in a 1 per cent dextrose broth, the action on this substance ceasing after 48 hours at 37° C., and sometimes as early as 18 hours, while allied

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species, regarded as the Colon group, go on uninterruptedly until the sugar is completely exhausted. In view of this peculiarity I believe it to be logical to substitute the name of "saccharolytic group" for these Colon-like organisms. I deem it unnecessary to go into details of the test for determining partial exhaustion of the sugar by *B. coli* and complete exhaustion of this substance by the saccharolytic group. This subject was thoroughly considered in the previous paper, and it suffices here to say that the test can be determined by the polariscope, or more practically by Fehling's Solution, as in testing diabetic urine.

It is not our purpose to go deeply into the exact chemical nature of the reactions, but more especially to determine the following points in regard to them:

1. *Relation of Test 2 to B. coli and the saccharolytic group.*—After exhaustive observations upon a number of cultures of true *B. coli* and those considered to belong to the saccharolytic group, I believe that my results demonstrate that Test 2 is characteristic of the former, and that any culture which does not show this reaction in spite of other biological characters, should be discarded as a true Colon and classified among the saccharolytes.

2. *Time required to obtain a positive reaction.*—To my satisfaction, it was found that Test 2 does not require the long wait of two to eight days required by the sulphuric acid and potassium nitrite test, but only one, or at the most two days of incubation at 37° C. In fact I have obtained this reaction on *B. coli* after six hours only, or in a shorter time, between four and five hours at 37° C., as soon as the slightest cloudiness of the medium was observed; this was never the case with the sulphuric acid and potassium nitrite test applied for comparison.

3. *Relation of Test 2 to the sulphuric acid and potassium nitrite test.*—As a matter of routine in studying a great number of cultures isolated from different sources and compared with a *B. coli* culture as control, both tests were applied simultaneously to the same culture, and the result corresponded very closely to the test for indol. In a few instances, however, Test 2 (sodium hydroxide and sulphuric acid) was positive, while the test for indol (sulphuric acid and potassium nitrite) remained negative, not because such cultures did not

produce indol, as the same thing was observed in the *B. coli* cultures, but because the test with sodium hydroxide and sulphuric acid was found to be more delicate and precise than the test with sulphuric acid and potassium nitrite, as determined by the following observation:

4. *Differences in Test 2 and indol test with different strength solutions of indol.*—Experiments with different strength solutions of indol crystals in distilled water were made and tested simultaneously by both tests. The sulphuric acid and potassium nitrite test gives a salmon-amber color, somewhat resembling the normal color of broth, while the sodium hydroxide and sulphuric acid give a bright purple-pinkish color decidedly more distinct than the sulphuric acid and potassium nitrite test.

5. *Delicacy of both tests.*—The sulphuric acid and potassium nitrite test was positive to the dilution of 1 : 1,000,000—that is, when the test was applied with all precautions and concentrated in forms of rings; it was almost indistinct in 1 : 800,000 when tested otherwise. Test 2 (sodium hydroxide and sulphuric acid) was found to be positive in 1 : 1,400,000, regardless of any precaution in making the test since this does not depend upon any concentration of the reactions but upon a diffuse general coloration of the medium. Further, to my satisfaction, by making the dilution with broth instead of distilled water, it was observed that the sodium hydroxide and sulphuric acid produced more or less destruction of the coloring matter of the medium, leaving an almost colorless broth upon which the reaction appears more pronouncedly, while the sulphuric acid and potassium nitrite produced no change in the color of the broth, which in some ways obscures the salmon-amber color of the reaction.

It was further noted that the color of the broth has an important bearing upon the sulphuric acid and potassium nitrite test, the darker the medium the less distinct being the reaction. Following this line of observation we found the test to be positive in some cases in the concentration of 1 : 500,000 only, and not beyond that point. Therefore for this reason, if for no other, Test 2 (sodium hydroxide and sulphuric acid) is preferable to sulphuric acid and potassium nitrite. Further it is not necessary to concentrate the reaction in the form of rings a method requiring a careful technic by no means always successful, but merely to add the sodium hydroxide and sul-

phuric acid without any special precaution. The coloration is a diffused bright purple-pink, of itself sufficiently distinct and characteristic.

Having determined by the above experiment that Test 2 (sodium hydroxide and sulphuric acid) bears a very close relation to the sulphuric acid and potassium nitrite test for indol, and having observed that this test is not only more delicate and in many ways superior and more easily performed, attention was next directed to determine if the meat-sugar-free broth made by the previous fermentation and exhaustion of the inosite in the meat juice during the incubation of 18 to 24 hours at 37° C., a method suggested by Smith and accepted by all bacteriologists, could be regarded as free from indol. It is stated that such preliminary fermentation by *B. coli* does not produce any perceptible amount of indol; however, in an effort to determine the correctness of both assertions, it was desirable to make some observations upon the subject. That indol is never produced in the presence of sugar is a well-known fact, but is it not possible that the amount of sugar present in the meat juice would be so small as to be easily exhausted by the *B. coli* in a few hours so that in the remaining time this organism would attack the proteid substances in the meat juice sufficiently to transform them into indol? Having determined that the sulphuric acid and potassium nitrite test for indol is not very delicate, and being in possession of Test 2 which showed itself to be more delicate and reliable, some experiments were conducted to determine the presence or absence of indol in meat-sugar-free broth.

Meat juice was tubed and sterilized in the autoclave at 20 pounds pressure for 20 minutes and a series of tubes inoculated with *B. coli* cultures (the amount inoculated was 1 drop of a 24-hour-old broth culture, this small amount being employed to avoid any possible error from the material transferred) and placed at 37° C. A number of tubes were tested by Test 2 after 2, 4, 6, 12, 18, 24, and 48 hours respectively. In some cases a positive reaction was obtained as early as after six hours' incubation. Most of the tubes showed a positive reaction after 12 hours, and this was more marked after 18 hours of incubation at 37° C.

Following the same line of experiments, the meat juice was tubed and without any preliminary sterilization, inoculated with *B. coli*

and incubated at 37° C. The test was applied as before, and the result was much the same. Further, with the idea that perhaps the subsequent sterilization would produce some changes in the indol occurring during the preliminary fermentation of the meat juice both experiments were repeated, but this time the test was applied after submitting some tubes to 100° C., and others to 20 to 30 pounds for 20 to 30 minutes. In both cases the heat was found to have had no effect on the reaction, as it was as typical and distinct as when no heat had been applied before performing the test, proving beyond any doubt that the subsequent sterilization, that is, the heat, has no effect on the reaction. This substantiated my experience in finding a positive reaction of indol in sterile broth control tubes, as well as in medium stored for laboratory use, and to this, no doubt, is due our recent literature on indol-positive typhoid strains.

As a matter of corroboration, and especially in order to determine in a more precise manner whether this substance was produced during the preliminary fermentation of the meat juice by *B. coli*, under exactly the same conditions the sulphuric acid and potassium nitrite indol test was applied in all the above experiments and the reactions were found to be negative after six hours. In one case only a very slight indol ring was obtained; in a few instances the reaction was concentrated in the form of rings after 12 to 18 hours and usually this was positive after 24 hours of incubation at 37° C.; this proves beyond a doubt the presence of indol sometimes in the meat-sugar-free broth. It is not true that all meat-sugar-free broth contains indol. In some experiments I was unable to detect this substance, due probably to an excess of acidity in the meat juice or to unfavorable conditions under which the preliminary fermentation was carried on, or to some inactivity of *B. coli* itself, which inhibited its action on the proteid substance, and under such circumstances it is a question whether even the sugar has been exhausted from the juice and whether such a broth can be regarded as free from this substance. It is a question, I believe, if this preliminary fermentation be desirable in order to exhaust the sugar in the meat. If so I would recommend the use of the saccharolytic group which rapidly attacks the sugar and produces no indol. Such cultures can be easily isolated from water and be used with advantage

instead of *B. coli;* I use for the present some of these cultures isolated from water producing 80 to 100 per cent of gas in 24 hours, which under the most delicate test have given negative indol reactions.

Before concluding, it is my desire to state that Test 2, if it be not a test for indol, can be properly regarded as something very closely related to it. Inasmuch as it is characteristic of *B. coli* it seems justifiable to use it in determining the identity of this organism, even though it may not be the same as the indol test.

In conclusion, I think the points to be emphasized from the results are:

First, the ease with which the test may be applied.

Second, the distinct and characteristic color.

Third, its applicability after an incubation of 24 hours, whereas by the ordinary indol test, a culture of eight days is recommended. (In this laboratory, 48 hours' incubation is given with the most satisfactory results.)

Fourth, the reaction does not have to be concentrated in the form of a ring.

Fifth, the rapidity with which a culture may be identified as a true *B. coli.*

Sixth, B. coli should be discarded as an agent for exhausting the sugar in broth, and one of the saccharolytic group used instead.